

1278-Pos Board B229**Backscattering Interferometry: Seeing Membrane Proteins in a New Light**

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The interaction of membrane proteins with and within lipid membranes is vital to a plethora of cellular processes from the control of intracellular signaling by peripheral membrane proteins, to the activity of antimicrobial peptides and integral membrane proteins. Thus, quantifying the mechanism by which proteins interact with and within lipid membranes is essential to understanding their biological functions. However, many protein-lipid and protein-protein interactions remain poorly understood due to the difficulty in studying processes that occur at the membrane surface. There is thus a clear need for a sensitive, versatile, non-perturbing and physiologically-relevant biophysical method capable of measuring association constant and stability free energy of membrane proteins in a wide range of conditions and matrices.

Backscattering interferometry (BSI) is an analytical technique that can monitor and quantify molecular interactions through the detection of small changes in refractive index (RI) induced by molecular interaction. BSI offers many advantages over current techniques including: no need for label or surface immobilization, small sample sizes (1-2 μ L), low concentrations (pM to μ M), remarkable sensitivity, broad dynamic range for dissociation constant, and low cost. Here, the potential application of BSI in the field of membrane proteins is illustrated through three case studies: (1) Association of small peptides with lipid vesicles, (2) transmembrane helix dimerization, and (3) unfolding of integral membrane proteins. Our preliminary results suggest that BSI is amenable to the study of such systems.

1279-Pos Board B230**Membrane Insertion Depth and Curvature Sensing**

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Many cellular processes require specific membrane deformations and reorganization, including endocytosis and cell division. Recently it has been shown that the degree of membrane curvature regulates activity and localization for some proteins such as ArfGAP1 and SpoVM. The short peptide SpoVM is one of the first proteins to localize to the forespore during *Bacillus subtilis* sporulation by recognizing the slightly curved convex membranes. However, it is unclear how a 26-amino acid protein can recognize curvature, but the unique topology of SpoVM and the phospholipid bilayer provide some insight into this process.

The non-traditional amphipathic helical structure of SpoVM consists of a short helical region and a flexible N-terminal loop. The helix has a comparatively large non-polar surface, with a small polar surface. These features allow for insertion of the helix deep within the phospholipid bilayer while the relatively polar tail can extend out of the membrane. We will present *in vivo* and *in vitro* data of SpoVM variants in support of our hypothesis that SpoVM's ability to sense membrane curvature is critically dependent on successful deep membrane insertion.

1280-Pos Board B231**Crystal Structure of the Bacterial Aminoarabinose Transferase ArnT**

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ArnT is a protein located in the inner membrane of gram-negative bacteria and is responsible for modification of lipid A, a complex lipid acting as anchor point for the lipopolysaccharide (LPS) of the outer membrane. ArnT is a lipid glycosyltransferase, catalyzing the transfer of a modified arabinose moiety from undecaprenyl phosphate to lipid A. The addition of the arabinose moiety reduces the negative charge of the outer membrane and limits its interaction with cationic peptides such as polymyxin B, thus enabling bacteria to develop resistance to polymyxin class antibiotics.

We report here the crystal structure of ArnT from a gram-negative bacterium at 2.8 Å resolution. To our knowledge, this is the first lipid-to-lipid glycosyltransferase structure. It consists of a transmembrane domain with thirteen transmembrane helices and a periplasmic soluble domain. The overall fold is reminiscent of previously solved protein glycosyltransferases from bacteria and archaea. However, the structure of ArnT has unique features that are related to its function as a lipid glycosyltransferase. One such feature is a cavity located on the side of the protein, accessible to the membrane environment. We hypothesize that this cavity allows free diffusion of lipid A into the active site.

A partially-interpretable electron density likely corresponding to the donor lipid is visible in our datasets. We have utilized this density to place the donor lipid in the ArnT structure, thus allowing us to identify the location of the active site. Potential coordinating residues for the headgroup of the donor lipid, as well as other notable features of the protein, will be tested using a polymyxin resistance growth assay.

We will compare and contrast the ArnT structure with available protein glycosyltransferase structures in an effort to gain insights on the structural basis of the catalytic mechanism of ArnT.

1281-Pos Board B232**Distinct Membrane Association Modes Facilitate Co-Translational Protein Targeting**

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Deciphering the nature of protein interactions at the membrane surface is crucial to understanding the molecular mechanism, timing, and regulation of many essential cellular processes. The signal recognition particle (SRP) and its receptor (SR), which target the translating ribosomes to translocation machineries at the target membrane, provide an ideal model system for investigating the mechanism of membrane recruitment of protein machineries.

Here we show that FtsY, the E. coli SR, interacts with the membrane through two distinct modes. Using single-molecule techniques that directly observe membrane association of FtsY in a supported lipid bilayer environment, we were able to detect and differentiate between the previously identified stable association mode as well as a transient interaction mode that is also crucial for targeting. Transition between these two modes is regulated by the SRP. Functional assays show that switching between transient and stabilized modes upon SRP binding is critical for successful targeting. We propose that, in contrast to currently accepted models in which FtsY is anchored on the membrane and awaits SRP encounter, this receptor rapidly cycles between cytosol and membrane and actively searches for cargo-loaded SRP. This active search could provide a mechanism to enhance efficient and accurate membrane localization of the targeting complex.

1282-Pos Board B233**Using Fluorescent-Labeled Nanodiscs to Study Lipid Interactions with Yeast Cytochrome C**

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Cytochrome c (cyt c) is a positively charged protein (+8 at neutral pH) that interacts with the inner mitochondrial membrane, which is composed of a large fraction of negatively charged phospholipid, cardiolipin (CL) (-2 at neutral pH). The peroxidation of CL by cyt c initiates the intrinsic pathway of apoptosis. We are investigating the interaction between cyt c and CL and the cyt c-CL complex using nanodiscs (ND). ND are a discoidal phospholipid bilayer model system, for which size and shape are governed by two membrane scaffolding proteins (MSP) belt proteins. The lipid composition can be altered to represent native-like or other experimental conditions. ND preparations are more stable and consistent between preps when compared to liposomes. Fluorescent labeling of the MSP belt proteins provides an effective method to monitor cyt c-CL interactions without perturbing the formation of cyt c-CL complexes. The quenching of the emission from the fluorophore-labeled MSP belt protein by the cyt c heme group is used to measure the binding affinity of cyt c for cardiolipin. Quenching is also used to determine stoichiometry and higher order assembly of cyt c-CL complexes.

1283-Pos Board B234**Biophysics of α -Synuclein Induced Membrane Remodelling**

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α -synuclein is an intrinsically disordered protein whose aggregation is a hallmark of Parkinson's disease. In neurons, α -synucleins is thought to play important roles in mediating both endo- and exocytosis of synaptic vesicles through